



User manual Invisorb® Spin Blood Mini Kit

For genomic DNA purification from fresh, frozen or old human blood with common anticoagulants (EDTA, Citrate) as well from buffy coat and bone marrow



1031100x00



STRATEC Molecular GmbH, D-13125 Berlin

Instruction for the Invisorb® Spin Blood Mini Kit

The **Invisorb® Spin Blood Mini Kit** is the ideal tool, using the Invisorb® technology for a fast, efficient and simple manual isolation and purification of genomic DNA from max. 200 µl fresh, frozen or old human blood with common anticoagulants (EDTA, Citrate) as well from buffy coat and bone marrow (max. 30 µl). The purified DNA can be used for in-vitro diagnostic analysis.

The kit is also useful for isolation of genomic DNA from max. 200 µl of non human mammalian blood or from up to 25 µl non mammalian blood, e.g. birds or fishes.

The kit is neither validated for the isolation of genomic DNA from tissue, serum, plasma, synovial fluid and urine, nor from bacteria, stool sample, fungi, parasites or the purification of total RNA

The application of the kit for isolation and purification of viral DNA has not been evaluated.



Compliance with EU Directive 98/79/EC on *in vitro* medical devices.

Not for in vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.

Trademarks: Invisorb®, Registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The Invisorb® technology is covered by patents and patent applications: US 6,110,363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

Invisorb® is a registered trademark of STRATEC Biomedical AG.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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Kit contents of the Invisorb® Spin Blood Mini Kit

Store dissolved Proteinase K at –20°C!

Store all other kit components at room temperature (RT)!

	5 DNA extractions	50 DNA extractions	250 DNA extractions
Catalogue No.	1031100100	1031100200	1031100300
Lysis Buffer HL	2 ml	15 ml	60 ml
Binding Buffer HL	2 x 1 ml (ready to use)	4 ml (final volume 16 ml)	2 x 8 ml (final volume 2 x 32 ml)
Elution Buffer	2 ml	15 ml	60 ml
Proteinase K	for 250 µl working solution	for 1.1 ml working solution	for 5 x 1.1 ml working solution
Pre-Wash Buffer	15 ml (ready to use)	30 ml (final volume 60 ml)	80 ml (final volume 160 ml)
Wash Buffer	15 ml (ready to use)	2 x 18 ml (final volume 2 x 60 ml)	3 x 45 ml (final volume 3 x 150 ml)
RTA Spin Filter Set	5	50	5 x 50
RTA Receiver Tubes	15	3 x 50	15 x 50
1.5 ml Receiver Tubes	10	2 x 50	10 x 50
Manuals	1	1	1
Initial steps	Add 250 µl ddH₂O to Proteinase K, mix thoroughly and store at -20°C!		
	Add 12 ml 99.7% Isopropanol to the Binding Buffer HL. Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.		
	Add 1.1 ml ddH₂O to Proteinase K, mix thoroughly and store at -20°C!		
	Add 30 ml of 96-100% ethanol to Pre-Wash Buffer, mix thoroughly and always keep the bottle firmly closed!		
	Add 42 ml of 96 -100% ethanol to Wash Buffer, mix thoroughly and always keep the bottle firmly closed!		
	Add 24 ml 99.7% Isopropanol to each Binding Buffer HL. Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.		
	Add 1.1 ml ddH₂O to Proteinase K, mix thoroughly and store at -20°C!		
	Add 80 ml of 96-100% ethanol to Pre-Wash Buffer, mix thoroughly and always keep the bottle firmly closed!		
	Add 105 ml of 96 -100% ethanol to Wash Buffer, mix thoroughly and always keep the bottle firmly closed!		

Symbols

	Manufacturer
	Lot number
	Catalogue number
	Date of manufacture
	Expiry date
	Consult operating instructions
	Temperature limitation
	Do not reuse

Storage

All buffers and kit components of the **Invisorb® Spin Blood Mini Kit**, except dissolved **Proteinase K** should be stored at room temperature (RT) and are stable for 12 months under these conditions.
Room temperature (RT) is defined as range from 15 - 30°C.

Dissolved **Proteinase K** stored at –20°C is stable for 12 months, but repeated freezing and thawing should be avoided. Aliquotation and storage at – 20°C is recommended.

Pre-Wash Buffer and **Wash Buffer** charged with ethanol should be appropriately sealed and stored at room temperature.

If there are any precipitates within the provided solutions dissolve these precipitates by carefully warming up to room temperature (up to 30°C).

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **Invisorb® Spin Blood Mini Kit** for applications as described in this manual. Purchaser must determine the suitability of the Product for its particular use. Should any Product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, STRATEC Molecular will replace the Product free of charge.

STRATEC Molecular reserves the right to change, alter, or modify any Product to enhance its performance and design at any time.

In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **Invisorb® Spin Blood Mini Kit** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

If you have any questions or problems regarding any aspects of **Invisorb® Spin Blood Mini Kit** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact:

from Germany	+49-(0)30-9489-2901/ 2910
from abroad	+49-(0)30-9489-2907
or contact your local distributor .	

Intended use

The **Invisorb® Spin Blood Mini Kit**, is the ideal tool for a fast and convenient manual isolation and purification of genomic DNA from max. 200 µl fresh, frozen or old human and from max. 100 µl mammalian blood as well from buffy coat (max. 30 µl) or non mammalian blood (max. 25 µl). For reproducible and high yields appropriate sample storage is essential. The purified DNA can be used for in-vitro diagnostic analysis only.

Fresh or frozen whole blood treated with EDTA or citrate, *but not with heparin*, from common blood collection systems can be used.

The protocol for the isolation and all buffers are optimized for a high yield as well as a high purity. All hands on steps are reduced to a minimum.

THE PRODUCT IS INDENTED FOR USE BY PROFESSIONAL USERS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

The kit is in compliance with EU Directive 98/79/EC on in vitro medical devices. But it is not for in vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.

Product use limitation

The kit is neither validated for the isolation of genomic DNA from tissue, serum, plasma, synovial fluid and urine, nor from bacteria, stool sample, fungi, parasites or the purification of total RNA

The included chemicals are only useable once.

Differing of starting material or flow trace may lead to inoperability; therefore neither a warranty nor guarantee in this case will be given, neither implied nor express.

The user is responsible to validate the performance of the STRATEC Molecular Product for any particular use. STRATEC Molecular does not provide for validation of performance characteristics of the Product with respect to specific applications. STRATEC Molecular Products may be used e.g. in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory the laboratory has been validated pursuant to CLIA' 88 regulations in the U.S. or equivalents in other countries.

All Products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and the plastic parts are for laboratory use only; they must be stored in the laboratory and must not be used for purposes other than intended.

The Product with its contents is unfit for consumption.

Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at www.stratec.com for each STRATEC Molecular Product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES, AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the liquid waste generated by the **Invisorb® Spin Blood Mini** procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and be handled and discarded according to local safety regulations.

European Community risk and safety phrases for the components of the **Invisorb® Spin Blood Mini Kit** to which they apply, are listed below as follows:

Lysis Buffer HL:



warning

H315-319-334-335 P280-305-351-310-405

Proteinase K:



danger

H225-319-336 P210-233-305-351-338

H315:	Causes skin irritation.
H319:	Causes serious eye irritation.
H225:	Highly flammable liquid and vapour.
H319:	Causes serious eye irritation.
H336:	May cause drowsiness or dizziness.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338:	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P210:	Keep away from heat/sparks/open flames/hot surfaces. — No smoking.
P233:	Keep container tightly closed.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA: 1 – 352 – 323 – 3500
in USA : 1 – 800 – 535 – 5053

Product characteristic of the Invisorb® Spin Blood Mini Kit

Starting material	Yield	Time for preparation	Ratio
1 - 200 µl fresh, frozen or old human or other mammalian whole blood (EDTA, citrate) 1 – 30 µl buffy coat 1 – 25 µl fresh, frozen or old non mammalian blood 1 - 20 µl bone marrow	up to 10 µg (in average about 6 µg) depends on amount of lymphocytes, sample source, sample transport, sample storage, and age of the sample	approx. 25 min	$A_{260} : A_{280}$ 1.7 – 2.0

The **Invisorb® Spin Blood Mini Kit** provides a very efficient procedure for isolation of high quality DNA directly from fresh, frozen, or old blood samples treated with citrate or EDTA or buffy coat samples.

The kit is designed for simultaneous processing of multiple samples. Prior separation of leukocytes is not necessary.

The whole blood sample is lysed in an optimized lysis buffer and proteins are degraded during the lysis with **Proteinase K**. The DNA binds to filter membrane, followed by washing steps and the final elution.

The purification procedure is rapid and requires neither phenol / chloroform extraction nor alcohol precipitation, and requires minimal interaction by the user, allowing safe handling of potentially infectious samples. The procedure is designed to avoid sample-to-sample cross-contamination. Due to the high purity, the isolated genomic DNA is ready to use for a broad panel of downstream applications (see below) or can be stored at –20°C for subsequent use.

Downstream Application:

- PCR *)
- Restriction Enzyme Digestion
- SNP Analysis
- HLA typing
- Cloning

To purify genomic DNA in 96 format STRATEC Molecular offers the **Invisorb® Blood Mini HTS 96 Kit** for use in a centrifuge and on common laboratory automated workstations. Furthermore STRATEC Molecular offers the **InviMag® Blood Mini Kits** for DNA isolation using magnetic beads.

To purify genomic DNA from large volumes of blood STRATEC Molecular offers the **Invisorb® Spin Blood Midi Kit** (max. 2 ml), the **Invisorb® Spin Blood Maxi Kit** (max. 10 ml), the **Invisorb® Blood Universal Kit** (1 – 10 ml).

For blood stains STRATEC Molecular offers the **Invisorb® Spin Forensic Kit**.

For further information please contact: Tel.: +49 (0) 30 9489 2901, 2910 in Germany and from foreign countries Tel.: +49 (0) 30 9489 2907 or your local distributor.

*) The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

Principle and procedure

The **Invisorb® Spin Blood Mini Kit** procedure comprises following steps:

1. lysis of sample material
2. binding the genomic DNA to the membrane of RTA Spin Filter
3. washing the membrane and elimination of ethanol
4. elution of genomic DNA

This manual contains 3 protocols, according to the different requirements of the starting materials.

Sampling and storage of starting material

Blood and Buffy Coat

Mammalian blood samples (stabilized with EDTA or Citrate) can be stored at room temperature for 2 - 3 hours, for short time storage (up to 24 h) samples may be stored at – 4 °C. For long term storage, we recommend freezing samples at – 20°C or – 80°C. Multiple thawing and freezing before isolating the DNA should be avoided. If cryoprecipitate (formed during thawing of frozen samples) are visible avoid aspirating them, they could clog the RTA Spin Filter membrane. Various different primary tubes, blood collection system (e.g. Sarstedt, Greiner) and anticoagulants (except heparin) can be used to collect blood samples for the **Invisorb®** procedure.

Buffy coat is a whole-blood fraction of enriched leukocyte cells. To prepare and extract a buffy coat layer the following procedure is recommended. The use of a whole blood sample (anticoagulants: EDTA, citrate, *not heparin*) with a sedimented cellular fraction from staying overnight at 4°C is recommended. The resulting bright mid-section overlaid by the clear plasma is buffy coat containing concentrated leukocytes that can be easily distinguished from the erythrocytes in the bottom layer. An enrichment factor of 10 is expected from such a procedure. Due to the enriched leukocyte content be aware to avoid overloading the DNA purification procedure.

Bone Marrow:

Best results are obtained with fresh material. It can be stored for 2-3 h at 4°C, for longer storage freeze the sample at –20°C. But often the sample will be dried. The have to be stored cooled at 4°C in a dry surrounding.

Procedure

Lysis

Samples are lysed at elevated temperatures. Lysis is performed in the presence of **Lysis Buffer HL** and **Proteinase K**.

Binding genomic DNA

By adding **Binding Buffer HL** to the lysate, optimal binding conditions will be adjusted. Each lysate is then applied to an RTA Spin Filter and genomic DNA is adsorbed to the membrane.

Removing residual contaminations

Contaminants are efficiently washed away using **Pre-Wash Buffer** and **Wash Buffer**, while the genomic DNA remains bound to the membrane.

Elution

Genomic DNA is eluted from the RTA Spin Filter using 30 - 200 µl **Elution Buffer**. The eluted DNA is ready for use in different downstream applications. Eluted DNA stored at 4 – 8°C is stable for 2 months, for more than 5 years if stored at -20°C.

Yield and quality of genomic DNA

The amount of purified DNA using the **Invisorb® Spin Blood Mini Kit** procedure depends on the sample type and the number of cells in the sample (depending from the patient's age and health situation, sample source, transport conditions, storage, and age of the sample).

Typically, a 200 µl sample of whole blood cells from a healthy individual will yield 3–10 µg of DNA. (If higher yields are required, use **Invisorb® Spin Blood Midi Kit** or **Invisorb® Spin Blood Maxi Kits** with up to 2 ml or up to 10 ml blood, respectively). Samples with elevated white blood cell (WBC counts, ranging from 3×10^6 to 1×10^7 cells/ml) give a higher yield.

For most whole blood samples, a single elution with 200 µl **Elution Buffer** is sufficient. For samples with elevated white blood cell approximately 80% of the DNA will elute in the first 200 µl, and up to 20% more in the next 200 µl.

Yield and quality of isolated genomic DNA is suitable for any molecular-diagnostic detection system. The diagnostic tests should be performed according to manufacturers' specifications.

Important points before starting a protocol

Immediately upon receipt of the Product, inspect the Product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to "Safety Information" (see page 7). Do not use damaged kit components, since their use may lead to poor kit performance.

- Always change pipet tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips.
- All centrifugation steps should be carried out at room temperature.
- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Discard gloves if they become contaminated.
- Do not combine components of different kits unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.
- This kit should only be used by trained personnel.

Preparing reagents and buffers

1. Adjust the thermomixer to 56°C.
2. Warm up the needed amount of **Elution Buffer** to 56°C (100 - 200 µl **Elution Buffer** are needed per sample).
3. Label the needed amount of RTA Spin Filter (lid).
4. Label the needed amount of 1.5 ml Receiver Tubes (per sample: 1 Receiver Tube).
5. Add the needed µl ddH₂O to reaction tube with **Proteinase K** (see below). Vortex for 5 s.
6. Add the needed amount of ethanol to the **Pre-Wash Buffer** and **Wash Buffer**.

5 DNA extractions:

add 250 µl dd H₂O to **Proteinase K**, mix thoroughly (vortex 5s) and store at –20°C
Binding Buffer HL, **pre-Wash Buffer** and **Wash Buffer** are ready to use

50 DNA extractions:

add 12 ml 99.7% Isopropanol to the **Binding Buffer HL**. Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.

add 1.1 ml dd H₂O to **Proteinase K**, mix thoroughly (vortex 5s) and store at -20°C

add 30 ml of 96 - 100 % ethanol to **Pre-Wash Buffer**

add 42 ml of 96 - 100 % ethanol to **Wash Buffer**, mix thoroughly and always keep the bottle firmly closed

250 DNA extractions:

add 24 ml 99.7% Isopropanol to each **Binding Buffer HL**. Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.

add 1.1 ml dd H₂O to **Proteinase K**, mix thoroughly (vortex 5s) and store at -20°C

add 80 ml of 96 - 100 % ethanol to **Pre-Wash Buffer**

add 105 ml of 96 - 100 % ethanol to **Wash Buffer**, mix thoroughly and always keep the bottle firmly closed

Reagents and equipment to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at www.stratec.com under each STRATEC Molecular kit and kit component.

- Microcentrifuge
- Thermomixer (for 56°C)
- Measuring cylinder (250 ml)
- Disposable gloves
- Pipette and pipette tips
- Vortexer
- Isopropanol*
- Reaction tubes (1.5 ml or 2.0 ml)
- dd H₂O
- 96 - 100 % ethanol
- 1 x PBS (optional)

*The **Invisorb® Spin Blood Mini Kit** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth**

* Possible suppliers for Isopropanol:

Carl Roth

2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem

2-Propanol für die Molekularbiologie
Order no. A3928

Sigma

2-Propanol
Order no. 59304-1L-F

Important indications

1. Process only as much blood samples as the microcentrifuge allows to process.
2. Blood sample and buffers should be thoroughly mixed and should have room temperature
3. The elution can be done by using lower amount of **Elution Buffer**. This may result in a higher concentration of DNA. But pay attention that minimum volume for elution is 30 µl, but this will reduce the yield. Elution volume between 2 x 30 µl up to 200 µl will realize comparable results.
4. The eluted DNA volume can be lower than the added **Elution Buffer** volume.
Elution Buffer should be preheated to 56 °C.
5. The **Elution Buffer** doesn't contain EDTA.
6. The yield can be increased if the incubation time with preheated **Elution Buffer** will be prolonged.
7. Old blood samples often contains coagulates, if coagulates or cryoprecipitate (formed during thawing of frozen samples) are visible avoid aspirating them, they could clog the Spin Filter membrane.

Scheme of the Invisorb® Spin Blood Mini Kit

 genomic DNA	Please read protocols prior the start of the preparation carefully Transfer max. 200 µl of the blood into a 1.5 ml Receiver Tube Add 200 µl Lysis Buffer HL and mix 5 times by pipetting up and down Incubate for 3 min at 56°C while continuously shaking Add 20 µl Proteinase K and vortex shortly. Incubate for 5 min at 56°C while continuously shaking Add 200 µl Binding Buffer HL (<i>follow preparing instructions</i>) and mix by pipetting up and down four times or vortexing Take a RTA Spin Filter Set Transfer lysate onto RTA Spin Filter Centrifuge for 2 min at 11.000 x g (11.000 rpm) Discard the filtrate and the RTA Receiver Tube Transfer the RTA Spin Filter in a new RTA Receiver Tube Add 500 µl Pre-Wash Buffer Centrifuge for 1 min at 11.000 x g (11.000 rpm) Discard the filtrate and the RTA Receiver Tube Place RTA Spin Filter to a new 2.0 ml RTA Receiver Tube Add 700 µl Wash Buffer Centrifuge for 1 min at 11.000 x g (11.000 rpm) Discard the filtrate and the RTA Receiver Tube repeat the step, but add the RTA Spin Filter to the same RTA Receiver Tube then centrifuge for 4 min at maximum speed for ethanol removal Place the RTA Spin Filter into a 1.5 ml Receiver Tube Add 100 - 200 µl of Elution Buffer (preheated to 56°C) Incubate for 1 min at room temperature Centrifuge for 1 min at 11.000 x g (11.000 rpm) Discard RTA Spin Filter Close the 1.5 ml Receiver Tube and store the DNA sample at 4 °C
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Protocol 1: DNA Isolation from 1 - 200 µl human and mammalian whole blood or 1 – 30 µl buffy coat

Please read the instructions carefully and conduct the prepared procedure.

Attention: Please be aware, that you have to prepare the **Binding Buffer HL** – see instruction page: 9

Important Transfer the needed amount of **Elution Buffer** into 2.0 ml Receiver Tube (not included in the kit) and place the tube at 56°C.

1. Transfer 1 - 200 µl whole blood or 1 – 30 µl buffy coat into a 1.5 ml reaction tube. If sample volume is lower than 200 µl, equilibrate with 1 x PBS Buffer or distilled water to 200 µl.
2. Add 200 µl **Lysis Buffer HL**, mix by pipetting up and down (5 times) and incubate for 3 min at 56°C while continuously shaking. Add 20 µl **Proteinase K** and vortex shortly.
3. Incubate the reaction tube for 5 min at 56°C while continuously shaking on a thermomixer.

Note: If you should use a water bath, please vortex the sample during lysis 2 – 5 times.

4. Add 200 µl **Binding Buffer HL** and mix the sample by vortexing or pipetting up and down for 4 - 5 times. Take a RTA Spin Filter Set. Transfer the mixture into the RTA Spin Filter. Close the RTA Spin Filter and incubate for 1 min.
5. Centrifuge for 2 min at 11.000 x g (11.000 rpm). Discard the filtrate and place the RTA Spin Filter in a new 2.0 ml RTA Receiver Tube.
6. Add 500 µl **Pre-Wash Buffer** to the RTA Spin Filter. Close the RTA Spin Filter. Centrifuge for 1 min at 11.000 x g (11.000 rpm). Discard the filtrate and place the RTA Spin Filter in a new RTA Receiver Tube.
7. Add 700 µl **Wash Buffer** and centrifuge for 1 min at 13.000 x g (12.000 rpm). Discard the filtrate and place the RTA Spin Filter in a new RTA Receiver Tube.
8. Add 700 µl **Wash Buffer** and centrifuge for 1 min at 11.000 x g (11.000 rpm). Discard the filtrate.
9. Place the RTA Spin Filter again into the 2.0 ml RTA Receiver Tube. Centrifuge for 4 min at maximum speed to eliminate the ethanol completely.
10. Place the RTA Spin Filter in a new 1.5 ml Receiver Tube. Add 200 µl of the preheated (56°C) **Elution Buffer**. Incubate at room temperature for 1 min.
11. Centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the RTA Spin Filter.

Note: The DNA can also be eluted with a lower volume of Elution Buffer (depends on the expected yield of genomic DNA). But pay attention that the minimum volume for the elution is **30 µl** and **that this volume can reduce the maximum yield**. If quite large amount of DNA is expected, the volume of Elution Buffer can be increased.

Note: The centrifugation steps were made with the **Centrifuge 5415 D from Eppendorf**. The indicated **rpm amounts** are referring to this centrifuge.

Protocol 2: DNA Isolation from non mammalian blood sample material

If you want to use bird (e. g. chicken) or fish blood that contain nucleated erythrocytes, the use of only 10-15 µl of starting material is recommended.

Please read the instructions carefully and conduct the prepared procedure.

Attention: Please be aware, that you have to prepare the **Binding Buffer HL** – see instruction page: 9

Important Transfer the needed amount of Elution Buffer into a 2.0 ml Receiver Tube (not included in the kit) and place the tube at 56°C.

1. Transfer 1 - 25 µl whole blood into a 1.5 ml reaction tube. Equilibrate with 1 x PBS Buffer e.g. to 200 µl.
2. Add 200 µl **Lysis Buffer HL**, mix by pipetting up and down (5 times) and incubate for 3 min at 56°C while continuously shaking. Add 20 µl **Proteinase K** and vortex shortly.
3. Incubate the reaction tube for 5 min at 56°C while continuously shaking on a thermomixer.

Note: *If you should use a water bath, please vortex the sample during lysis 2 – 5 times.*

4. Add 200 µl **Binding Buffer HL** and mix the sample by vortexing or pipetting up and down for 4 - 5 times. Take a RTA Spin Filter Set. Transfer the mixture into the RTA Spin Filter. Close the RTA Spin Filter and incubate for 1 min.
5. Centrifuge for 2 min at 11.000 x g (11.000 rpm). Discard the filtrate and place the RTA Spin Filter in a new 2.0 ml RTA Receiver Tube.
6. Add 500 µl **Pre-Wash Buffer** to the RTA Spin Filter. Close the RTA Spin Filter. Centrifuge for 1 min at 11.000 x g (11.000 rpm). Discard the filtrate and place the RTA Spin Filter in a new RTA Receiver Tube.
7. Add 700 µl **Wash Buffer** and centrifuge for 1 min at 11.000 x g (11.000 rpm). Discard the filtrate and place the RTA Spin Filter in a new RTA Receiver Tube.
8. Add 700 µl **Wash Buffer** and centrifuge for 1 min at 11.000 x g (11.000 rpm). Discard the filtrate.
9. Place the RTA Spin Filter again into the 2.0 ml RTA Receiver Tube. Centrifuge for 4 min at maximum speed to eliminate the ethanol completely.
10. Place the RTA Spin Filter in a new 1.5 ml Receiver Tube. Add 200 µl of the preheated (56°C) **Elution Buffer**. Incubate at room temperature for 1 min.
11. Centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the RTA Spin Filter.

Note: *The DNA can also be eluted with a lower volume of Elution Buffer (depends on the expected yield of genomic DNA). But pay attention that the minimum volume for the elution is **30 µl** and that this volume can reduce the maximum yield. If quite large amount of DNA is expected, the volume of Elution Buffer can be increased.*

Note: *The centrifugation steps were made with the **Centrifuge 5415 D** from Eppendorf. The indicated **rpm amounts** are referring to this centrifuge.*

Protocol 3: DNA isolation from CFS and bone marrow

For the isolation and purification of DNA from small amounts of various human and mammalian samples

Please read the instructions carefully and conduct the prepared procedure!

Attention: Please be aware, that you have to prepare the **Binding Buffer HL** – see instruction page: 9

Important Transfer the needed amount of Elution Buffer into an 2.0 ml Receiver Tube (not included in the kit) and place the tube at 56°C.

Preparation of the starting material:

Fresh material:

- 1 – 20 µl bone marrow

Dried material (for example on hematological slides):

- Moisten the dried material with a drop of PBS.
- Add 180 µl PBS to a 1.5 ml Receiver Tube (not provided).
- Scrape cytological material into the Receiver Tube using the edge of a clean slide.
- Dissolve the resulting sludge by pipetting up and down.

Sample Lysis

1. Transfer the starting material into a 1.5 ml reaction tube. Equilibrate with 1 x PBS Buffer e.g. to 200 µl.
2. Add 200 µl **Lysis Buffer HL**, mix by pipetting up and down (5 times) and incubate for 3 min at 56°C while continuously shaking. Add 20 µl **Proteinase K** and vortex shortly.

Important: Vortex the sample for 10 sec! An incomplete mixing will reduce quality and yield of the isolated DNA.

3. Incubate the reaction tube for 5 min at 56°C while continuously shaking on a thermomixer.

Note: If you should use a water bath, please vortex the sample during lysis 2 – 5 times.

Proceed as described in protocol 1 steps 4 – 11.

Troubleshooting

Problem	Cause	Comments and suggestions
low amount of DNA	insufficient cell lysis	increase lysis time with Lysis Buffer HL reduce amount of starting material continuously shaking improves lysis efficiency
	insufficient cell lysis due to decreased Proteinase K activity	repeat the DNA purification procedure with a new sample and freshly prepared Proteinase K stock solution, be sure to store the stock solution at -20°C ensure, that Proteinase K is not added directly to Lysis Buffer HL
	insufficient lysis due to insufficient mixing with Lysis Buffer HL	repeat the DNA purification procedure with a new sample. be sure to mix the sample and Lysis Buffer HL immediately and thoroughly by pipetting up and down 5 times or by pulse-vortexing
	inefficient binding of DNA to the membrane	overloading RTA Spin Filter reduces yield - use correct amount of Binding Buffer HL - mix sample with Binding Buffer HL by pipetting up and down 4-5 times or by vortexing (5 sec) prior to transfer the sample onto the Spin Filter
	low percentage alcohol used instead of 96 - 100%	repeat purification procedure with a new sample
	incomplete elution	increase incubation time with preheated Elution Buffer to 5 - 10 min elute twice with each 100 μl Elution Buffer use higher volume of Elution Buffer .
	low DNA-concentration in the sample	elute the DNA with lower volume of Elution Buffer
	pH of water incorrect (acidic)	low pH may reduce DNA yield. Ensure that the pH of the water is at least 7.0 or use Elution Buffer (contains only 10 mM Tris – HCL, no EDTA)
colored residues remain on the RTA Spin filter after washing	insufficient cell lysis	see above
	no Binding Buffer HL added to the lysate before loading onto the RTA Spin Filter	repeat the purification procedure with a new sample
	inefficient washing	wash again with Wash Buffer
	Pre-Wash Buffer and Wash Buffer prepared incorrectly	ensure that Pre-Wash Buffer and Wash Buffer concentrates were diluted with the correct volume of pure ethanol. Repeat the purification with a new sample
	old material, repeated frozen material	old blood may lead to colored membrane of the RTA Spin Filter, this color will not be eluted during purification yield and quality of DNA is reduced based on sample storage
degraded or sheared DNA	incorrect storage of starting material	ensure the sample is harvested and stored as described on page 8
	old material	avoid repeated thawing and freezing of the material old material often contains degraded DNA

Problem	Cause	Comments and suggestions
clogged Spin Filter	incorrect storage of starting material insufficient lysis too much starting material	blood stored longer time at RT may form clumps of proteins etc. Prevent a transfer of this clumps into the sample cryoprecipitates have formed in blood due to related freezing and thawing, do not use blood that has been frozen and thawed more than once, prevent a transfer of cryoprecipitates into the sample perform isolation as described in protocol 2 increase lysis time with Lysis Buffer HL increase centrifugation time and/or speed reduce amount of starting material concentration of leukocytes in samples was greater than $5 \times 10^6 / 200 \mu\text{l}$
problems with subsequent applications (e.g. in PCR)	ethanol in the eluted DNA salt in the eluate reduced sensitivity of amplification reaction	verify if the recommended centrifugation time was reached increase centrifugation time for the elimination of ethanol if necessary Wash Buffer should be stored at and used at RT verify Wash Buffer on the precipitation of salt. If there are precipitations dissolve this by careful warming up to 30°C adjust the volume of eluate added as template in the amplification reaction
A₂₆₀/A₂₈₀ ratio for purified DNA is high	high level of residual RNA	in future DNA preparations prepare an RNase digestion step add 10 μl RNase A (10 mg/ ml) to the eluted DNA, vortex shortly and incubate 10 min at RT
A₂₆₀/A₂₈₀ ratio for purified DNA is low	insufficient cell lysis and protein degradation due to decreased Proteinase K activity insufficient lysis due to insufficient mixing with Lysis Buffer HL	see above under “low amount of DNA” see above under “low amount of DNA”

Appendix

General notes on handling DNA

Starting material

This kit is designed for extraction of DNA from blood, but even human blood is different between individuals depending on age, health, and conditions of life. If you are using blood from animals keep in mind that lyses conditions of blood differ depending on the species. Also remember that non-mammalian blood contains erythrocytes with nuclei. So for special applications adaptation of starting volumes and lyses time may be recommended.

Nature of DNA

The length and delicate physical nature of DNA requires careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure compatibility with various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting and long-template PCR.

Storage of DNA

A working stock of DNA can be stored at 2 – 4°C for several weeks. For long term storage DNA should be stored at -20°C, but storing at – 20°C can cause shearing, particularly if the DNA is exposed to repeated freeze-thaw cycles.

Note that the solution in which the nucleic acid is eluted in will affect it's stability during storage. Pure water lacks buffering capacity and an acidic pH may lead to acid hydrolysis. Tris or Tris-EDTA buffer contains sufficient buffering capacity to prevent acid hydrolysis.

Drying, dissolving and pipetting DNA

Avoid over drying genomic DNA after ethanol precipitation. It is better to let it air dry than to use a vacuum, although vacuum drying can be used with caution.

Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings causes shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA.

DNA Yield

The amount of purified DNA from the whole blood depends on the leucocytes content, sample source, transport, storage, and age. Various different primary tubes and anticoagulants (except heparin) can be used to collect blood samples for the **Invisorb®** procedure.

Ordering information

Product	Package size	Catalogue No.
Invisorb® Spin Blood Mini Kit	5 preparations	1031100100
Invisorb® Spin Blood Mini Kit	50 preparations	1031100200
Invisorb® Spin Blood Mini Kit	250 preparations	1031100300
Lysis Buffer HL	15 ml	1031101100
Binding Buffer HL	15 ml	1031102100
Pre-Wash Buffer (add 30 ml)	30 ml	1031103300
Wash Buffer (add 42 ml)	18 ml	1031103400
Elution Buffer	15 ml	1031104000
Invisorb® Spin Blood Midi Kit	50 preparations	1031110300
Invisorb® Spin Blood Maxi Kit	50 preparations	1031120300
Invisorb® Blood Universal Kit	500 ml	1031150200
Invisorb® Blood Universal Kit	1000 ml	1031150300
Invisorb® Blood Mini 96 HTS/ C	4 x 96 preparations	7031300300
Invisorb® Blood Mini 96 HTS/ C	24 x 96 preparations	7031300400
InviMag® Blood DNA Mini Kit/ KFml	15 preparations	2431110100
InviMag® Blood DNA Mini Kit/ KFml	75 preparations	2431110200
InviMag® Blood DNA Mini Kit/ KF96	1 x 96 preparations	7431300100
InviMag® Blood DNA Mini Kit/ KF96	5 x 96 preparations	7431300200

Possible suppliers for Isopropanol:

Carl Roth

2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem

2-Propanol für die Molekularbiologie
Order no. A3928

Sigma

2-Propanol
Order no. 59304-1L-F



STRATEC Molecular GmbH
Robert-Rössle-Str. 10
13125 Berlin, Germany

Phone: +49 30 94 89 29 01
Fax: +49 30 94 89 29 09
E-mail: info.berlin@stratec.com

www.stratec.com